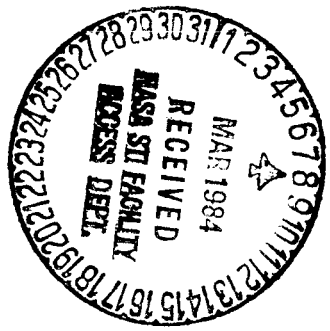
FLUID MECHANICAL ASPECTS OF CELL CULTURE



Final Report
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Donald L. Williams
President

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Objective

The research project had the objective of determining the influence of shear rate on cell cultures. Cells were cultured on a flat substrate in a specially designed flow chamber in which shear rate is known and controlled.

Equipment

The required items of equipment were obtained and placed in operation. The major items are listed below.

1. The Richardson Flow Chamber, consisting of a glass slide (where the cells are attached and grow), the main body of the flow chamber (which has two slits for entry and exit of flow and a connector for a vacuum line), and a rectangular silastic gasket with holes to transmit vacuum to the glass slide. The glass slide and the main body of the flow chamber are held parallel at a known spacing by the gasket. This provides a flow channel above the glass surface.

2. A double syringe continuous automatic infusion pump is used to yield a known, constant flow rate of culture medium through the flow chamber at various prescribed levels. The pump is operated with infusion from one syringe with simultaneous withdrawal into the other. In this way contamination during the operation is reduced.

3. An Air Curtain Incubator is used for temperature control. The detector of the incubator is placed on the slide's surface and controlled to 37.0°C $\pm$0.5.

Cell Culture Techniques

Human embryonic kidney cells lot #8514 were used in all experiments. Techniques were developed for (i) cleaning and treating the slide's surface prior to the cell transfer to facilitate cell attachment, (ii) for growing the cells on the slides, (iii) treating them for successful transfers to new slides, and (iv) for keeping the cell lines up to 4 or 5 passes while avoiding contamination.

Basic Experiments

The flow chamber was used with the cell slide attached to the bottom of the chamber providing a well defined area of cells exposed to the shear field. Experiments were performed for shear stresses in the range of 2 to 60 dynes/cm^2, at 37°C $\pm$0.5, with the time of exposure to the shear stresses varied between 2 and 24 hours.

Morphological analysis of the data was carried out by using a Zeiss-Mapping system from which the following information was obtained:

- Area: cell area (μ^2)

- Length: cell perimeter (μ)

- Count: number of cells traced

- Maximum Diameter: cell's maximum diameter (μ)

- Angle: the slope of maximum diameter from the center line (0^o to 360^o)

- Form: cell shape defined to be $(4)(\text{area})/\pi D^2 m$. A circular cell has a shape of 1; lower shape number indicates cell elongation.

The collected data are shown in Table I.

TABLE I. MORPHOLOGICAL RESULTS

TIME (HOURS	SHEAR STRESS (D/cm^2)	AREA (μ)		PERIMETER (μ)		NUMBER OF CELLS TRACED	MAXIMUM DIAMETER (μ)		FORM	
		M	S	M	S		M	S	M	S
CONTROL		215.33	87.34	66.05	13.81	372	24.00	6.94	0.6	0.12
2	2.60	235.94	112.72	70.12	18.26	484	25.63	8.70	0.6	0.12
2	6.54	315.57	153.26	82.63	22.54	415	30.02	10.02	0.6	0.13
2	13.00	305.98	151.70	83.63	21.88	176	31.62	10.61	0.6	0.14
2	26.00	378.09	193.57	103.80	30.02	271	44.35	14.51	0.5	0.04
2	54.00	454.82	207.14	121.70	34.24	119	50.03	15.01	0.4	0.11
4	2.60	296.31	108.70	74.58	17.89	189	27.53	8.76	0.6	0.12
4	6.54	333.55	117.94	89.67	23.07	145	37.49	11.41	0.6	0.03
4	13.00	389.83	184.56	108.43	34.59	73	47.45	16.93	0.4	0.14
4	26.00	401.53	193.45	118.99	30.09	113	51.13	15.39	0.4	0.10
4	54.00	499.55	222.80	143.96	46.38	72	56.84	15.96	0.4	0.02
8	6.54	345.47	159.73	98.89	25.62	153	41.94	13.20	0.5	0.07
8	13.00	385.90	130.09	107.48	24.55	153	49.21	12.12	0.4	0.13
8	26.00	448.90	206.88	127.80	35.26	22	54.60	15.73	0.4	0.14
8	52.00	533.22	149.53	148.94	42.96	79	58.51	15.20	0.3	0.12

M - mean value
S - mean standard deviation

These selected data and optical observation of the cell show that:

1. The influence of the shear field is slight at low shear stresses (2.60/dyn/cm^2). At higher shear stresses (above 26.00 dyn/cm^2) the cells lose their viability and tend to come off the glass surface.

2. At intermediate and high shear rates (between 6.54 and 54.00 dyn/cm^2 the cells tend to be oriented parallel to the direction of flow.

3. At intermediate and high shear rates (between 6.54 and 54.00 dyn/cm^2 the cells in the flow become significantly larger than control cells.

4. At intermediate and high shear rates (between 6.54 and 54.00 dyn/cm^2 cell elongation is observed causing a change in the cell form.

Metabolic Activity Measurements

Fibrin Overlay Methods for the detection of metabolic activity of colonies of transformed cells were evaluated. From these evaluations a method was selected (adapted from the procedure of Jones, et al) and developed for urokinase detection.

The results are given in Table II. The numbers indicate urokinase units produced per cell.

TABLE II.
INFLUENCE OF SHEAR FIELD ON UROKINASE PRODUCTION

TIME (HRS.)	CONTROL	SHEAR STRESS, DYNES/CM^2	
		2.60	26.00
CONTROL	0.36×10^{-4}		
2		0.31×10^{-4}	
4			0.14×10^{-4}

The data indicate a significant reduction of urokinase, released by the cells, at high shear rates.